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PRINCIPAL INVESTIGATOR: In Hong Yang

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore MD 21205

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In Hong Yang

E-Mail: iyang3@jhmi.edu

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14. ABSTRACT

From petri dish to complex micro-devices, technological advances in microfluidic devices allowed us to study various disease cases in greater depth. Multiple Sclerosis (MS) is an autoimmune disease which occurs in the brain. The inflammation during MS is also known to occur at the wall of the tissue where Neural Progenitor Cells reside (NPC). These NPCs are recognized for their regenerative property; they can replace old or damaged neurons with newly formed neurons. Thus, in MS patients, it is difficult to maintain neurogenesis for restorative therapy as it is constantly inhibited due to the inflammation. Pathological studies reveal that the two microenvironments surrounding the inflammation site are different. This calls for a novel microfluidic device that mimics distinct microenvironments of the disease condition. Hence, we have developed a three-compartment system microfluidic system that can be used to study such disease. Using this device, the cellular and molecular signaling mechanism under MS in the NPCs may be elucidated for the first time.

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Introduction: Neural stem cells (NSCs) are multipotent cells isolated from striatal tissue and the subventricular zone (SVZ), which is one of a few neurogenic areas in the adult brain. NSCs in the brain have the potential to remyelinate damaged axons and restore neural function after nerve injury. Recent progress in our understanding of the biology of NSCs has inspired interest in exploring the roles of neurogenesis capable NSC in the pathology and therapy of neurodegenerative disorders including multiple sclerosis (MS) [1-2]. Evidence suggests that NSC proliferation and differentiation occur under physiological conditions and can be enhanced in certain pathological conditions following neural damage [3-4]. We hypothesize that in MS, soluble mediators released by inflammatory T-cells cause abnormal proliferation and differentiation of NSCs resulting in the impairment of neurogenesis of the brain. In the MS brain, NSCs experience spatially and temporally asymmetric levels of fulminant attacks by inflammatory T-cells. However, the communication between soluble factors of inflammatory T cells and NSCs that affect the proliferation and differentiation of NSCs remain unknown. The goal of this project is to develop a novel NSC culture platform that is capable of both compartmentalizing and fluidically isolating microdomains of NSC neurospheres. By building such a platform, aspects of the molecular and cellular signaling between micro-populations of NSCs and inflammatory T cells/macrophages can be elucidated for the first time in a novel in vitro system

Body: Preliminary design was created using AutoCAD. To create a microfluidic device of our goal, two-layer design was implemented: one for separate compartments, and one for the micro-channels that connect between compartments and allow molecular signaling. The height and width of the microchannel is 10um, the width of the middle chamber (where cells will be plated) is 800um, and the distance between the compartments is 400um.

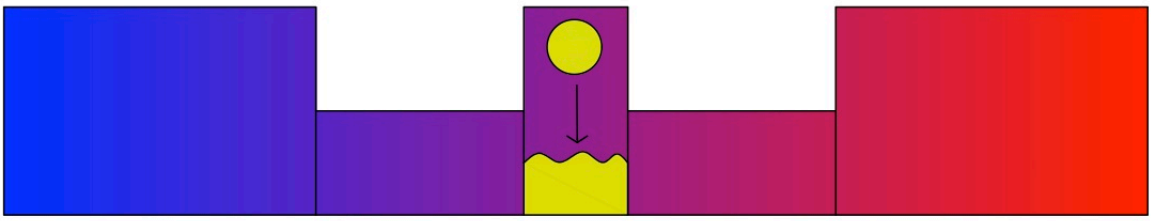

Figure 1. This is a 2D diagram of the device. Middle well is where the cells are plated. Microchannels connect the compartments.

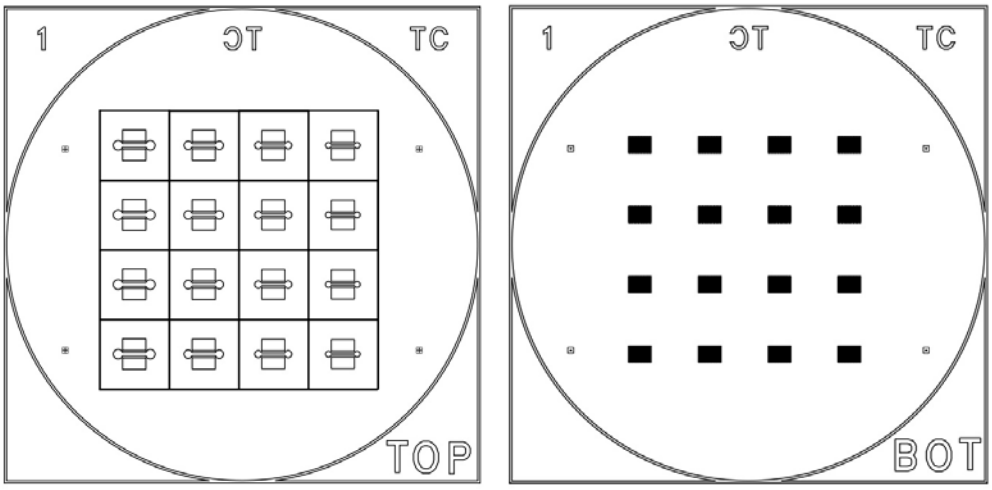

Figure 2. Left) layer for microchannels Right) layer for compartments. Cells are plated in the middle well which has circular ends and two compartments besides it mimic the pathological micro-environment.

Conclusion: Silicon wafer product was developed through soft lithography using Poly-Di-Methyl-Siloxane. Soft lithography technique is very useful in replicating the structures without causing damage to the original mold. PDMS is a superb material for developing micro-fluidic devices. The reason for such prevalent usage of PDMS is because it is inert, transparent, and easy to handle but at the same time robust. PDMS was mixed with curing agent and then degased to rid air bubbles. Then, it was poured on to the silicon wafer and individual device.

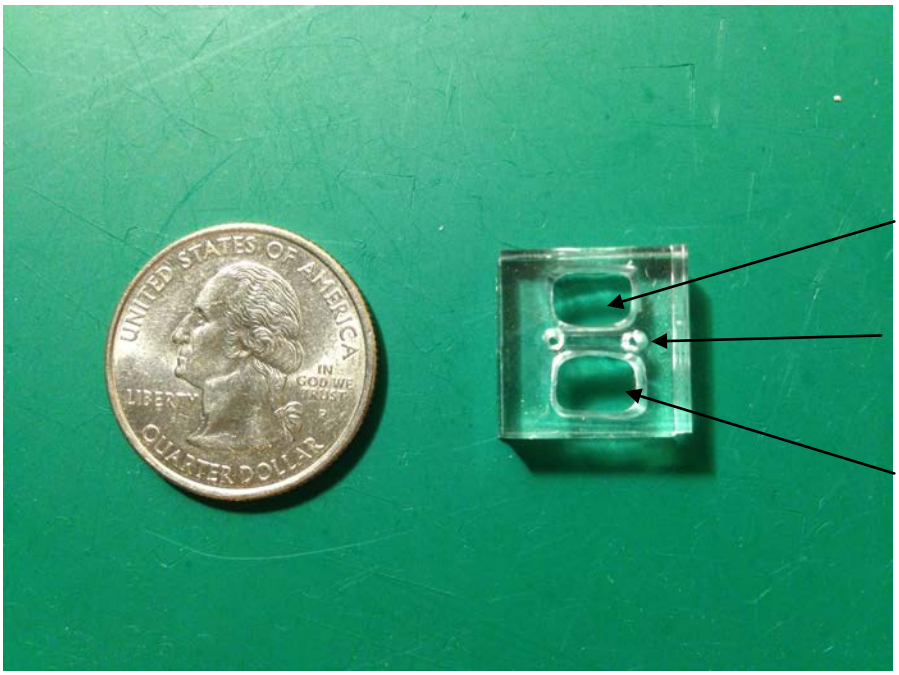

Figure 4. Actual size of the microfluidic device

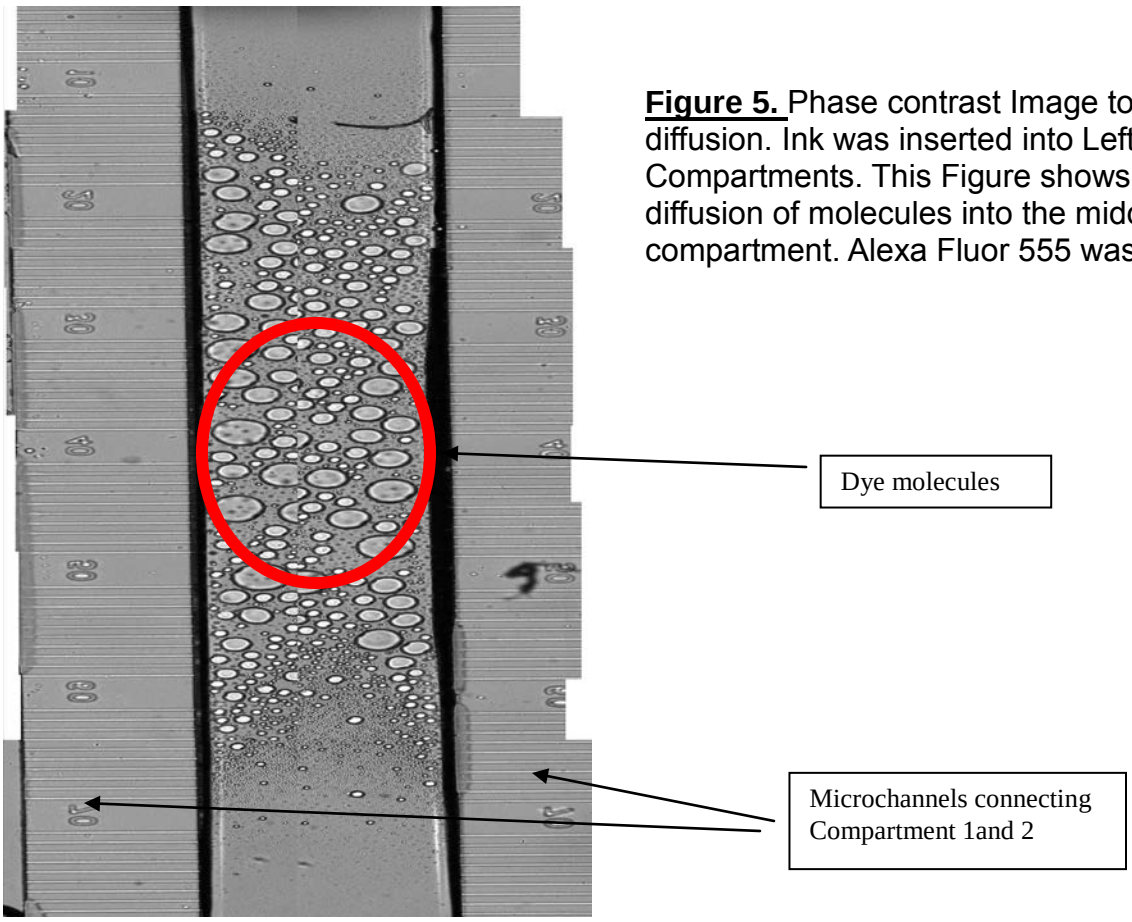

Figure 5. Phase contrast Image to confirm diffusion. Ink was inserted into Left and Right Compartments. This Figure shows the diffusion of molecules into the middle compartment. Alexa Fluor 555 was used.

A multi-compartmental system was successfully created. Using this chamber, NPCs will be cultured and exposed to pathological conditions of Multiple Sclerosis. Granzyme B is known to inhibit neurogenesis in MS. NPCs will be exposed to Granzyme B in one compartment and proliferating media on the other. Fluorescence imaging will be used to elucidate cellular signaling pattern.

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